sbeadex Pathogen Nucleic Acid Purification Kit

Protocol for manual extraction

For Research Use Only.
Not for use in diagnostic procedures.

1. Preparation of samples

This protocol has been verified for a range of sample matrices. These are sample swabs in viral transport medium (VTM), sputum, whole blood, serum, plasma, urine, stool and cerebrospinal fluid. Sputum was prepared following CDC guidelines.

2. Preparing the particle and buffer premix

The sbeadex™ particle suspension and Binding buffer SB can be added to the reaction(s) as a premix.

To prepare the premix for the sbeadex Pathogen Nucleic Acid Purification Kit protocol:

a. Thoroughly mix the sbeadex particle suspension to fully resuspend the particles
b. Add 20 µL sbeadex particle suspension to 160 µL Binding buffer SB.

If preparing premix for multiple reactions, multiply the volumes accordingly and allow sufficient overage for accurate pipetting.

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Protocol

3. Nucleic acid purification

3.1. Optional pre-lysis for bacterial samples

1. Add the following to the reaction tube/well in the order listed below:
   a. Optional: 20 µL Protease solution
   b. Optional: 1 µg carrier DNA/RNA
   c. 100 µL of the liquid starting sample
   d. 100 µL (1x) Lysis buffer SB
2. Incubate at 55 °C for 10 minutes with constant shaking.
3. Allow the sample(s) to cool to room temperature.
4. Proceed to the step-by-step protocol (section 3.2).

NOTE: Some bacterial species may require further treatment (i.e. heat inactivation at 90 °C and/or zirconium beads) to disrupt the cell wall.

3.2. Step-by-step manual protocol for nucleic acid purification post lysis

1. Add 20 µL sbeadex particle suspension and 160 µL Binding buffer SB (these can be added as a 180 µL of premix – see section 2).
2. Mix thoroughly and incubate for 10 minutes at room temperature with constant shaking.
3. Bring magnet into contact with the tube(s) for 2 minutes.
4. Remove the supernatant and discard.
5. Separate the magnet from the sample tube(s).
6. Add 400 µL Wash buffer BN1.
7. Incubate for 5 minutes at room temperature with constant shaking.
8. Bring magnet into contact with the tube(s) for 2 minutes.
9. Remove the supernatant and discard.
10. Separate the magnet from the sample tube(s).
11. Repeat steps 6-10 with Wash buffer TN1.
12. Repeat steps 6-10 with Wash buffer TN2.
13. Add 100 µL Elution buffer AMP. Mix thoroughly.
14. Incubate for 10 minutes at 60 °C with periodic shaking.
15. Bring magnet into contact with the tube(s) for 3 minutes.
16. Transfer the nucleic acid-containing eluate to a new tube by pipetting, avoiding the transfer of any sbeadex beads.

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Protocol

4. Safety information

To access the SDS document for the components in this kit, please visit our website. Work with infectious virus should be carried out according to the regulation of the country within which the kit is being used.

- Wear appropriate skin and eye protection throughout the preparation procedure.
- Lysis buffer SB, Binding buffer SB and Wash buffer TN1 contain high concentrations of detergent and salt.
- Binding buffer SB and Wash buffer TN1 contain up to 50% n-propanol, therefore keep away from naked flames.
- Ensure kit components are stored appropriately according to local safety guidance.
- In case of accidental contact, thoroughly rinse or flush the affected areas with water.
- Spillages can be removed using standard laboratory cleaning procedures.
- Safety data sheets are available for all kit components on request.

Kit component	GHS symbol	Hazard phrases	Precaution phrases
Lysis buffer SB	 Warning	H302/H315/H319/H400	P101/P102/P103/P273/ P280/P305+P351+P338/ P301+P312/P332+P313/P501/ P301+P312
Protease solution	Danger 	H334/H317	P101/P102/P103/P261/ P304+P341/P501
Binding buffer SB	 Danger	H226/H302/H315/H318/H336/H400	P101/P102/P103/P210/ P241/P303+P361+P353/ P305+P351+P338/P310/P501
sbeadex particle suspension	-	-	-
Wash buffer BN1	 Danger	H226/H332/H315/H318/H336	P101/P102/P103/P210/ P303+P361+P353/ P305+P351+P338/P310/ P405/P501
Wash buffer TN1	 Danger	H315/H318/H226/H336	P101/P102/P103/P210/ P303+P361+P353/ P305+P351+P338/P310/P405/ P501
Wash buffer TN2	-	-	-
Elution buffer AMP	-	-	-

Table 1. Safety information for sbeadex Pathogen Nucleic Acid Purification Kit components

5. Technical support

If you require additional information or technical assistance, please feel free to email our Technical Support Team at: techsupport@lgcgroup.com.

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